PROJECT REPORT

FOR

BIOFERTILIZER LABORATORY AND PRODUCTION UNIT

PREPARED FOR
BIOMATE INDIA
DELHI

PREPARED BY,
BIOMATE INDIA
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PROMOTERS PROFILE

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TECHNICAL DETAILS

Introduction:
In past 50 years history, the chemical pesticides and fertilizers have played a crucial role in boosting the agricultural production, however they have a short history in modern agriculture. Their immediate action and low cost managed to bring them rapidly in to the center of attention. Their toxic effects on environment, plant, animal and human life diverted the focus on ecofriendly plant protection. Moreover, the development of resistance in insects against common pesticides has not been solved yet. Thus, practices such as Integrated Pest Management (IPM) have gained more importance. Biopesticides are important component of the IPM. The terminology 'Biopesticide' means use of beneficial microorganisms to control the pest. However, the availability of biopesticide is the major constraint; considering the total cropped area.

Indiscriminate use of chemical pesticides contributed in loss of soil productivity along with addition of salts to the soil. To revive the soil health and living on alternate source has become essential concept of biofertilizer came forward, which can be a good supplement for a chemical fertilizers. Biofertilizers are nutrient availability systems in which biological process are involved. The term biofertilizers includes selective micro-organism like bacteria, fungi and algae. Which are capable of fixing atmospheric nitrogen or convert soluble phosphate and potash in the soil into forms available to the plants. Biofertilizer is a cost effective, eco-friendly & renewable source of land nutrient they play a vital role in maintaining a long term soil fertility & sustainability.

The biofertilizer with nitrogen fixer & phosphate solubilizer fixes 20-40 Kg of nitrogen per acre. The biofertilizer maintain the soil fertility cost by using in the yield is assured with biofertilizer & continuos use of biofertilizer makes the soil very fertile for good yield. The biofertilizer can be manufacture in soil form or in liquid form for spraying on the plants.

Biopesticide and biofertilizer is a need of modern agriculture since demand for safe and residue free food is increasing. Therefore, to cater the need, it is necessary to promote the efforts for production of biopesticides and biofertilizers in the state in private sector to encourage the entrepreneurs.

Market status and Scope:
Sustainable agricultural is a complex issue associated with producing food while maintaining biophysical resources including soil, water & biota with no adverse impact on the wider environment. Biofertilizer and biopesticide fulfills all the needs for sustainable agriculture.

In spite of the fact that biopesticides have been extensively used in developed countries for the past few decades, the non-availability of the same products suitable for the regionally-important pests resulted in the sluggishness of the growth of biopesticides in developing countries. However, in recent years, awareness on biopesticides among farmers of the developing world has been increasing with newer products entering the market. And surprisingly, most currently registered products for insect control are produced in developing countries and included bacteria (104 products), nematodes (44 products), fungi (12 products), viruses (8 products) and protozoa (6 products). Market for biopesticides is increasing as the agrochemical industry adapts to the problems of chemical pesticide resistance, re-registration of pesticide and changing public attitudes to pesticide use.

In India, similar condition is for biofertilizer production. Biofertilizer are available for almost all crops for three nutrient ie. N (N-fixers), P (P-solubilizers) & K (K-mobilizers). Therefore biofertilizer consumption is expected to increase too many fold in coming years. Previously the state agriculture universities non-government organizations were engaged in mass production of biofertilizers. But now several small and medium sector manufacturers have come in picture. Largest producing states being Madhya Pradesh, Maharashtra, Karnataka and Tamilnadu. The biofertilizer demand in India is several thousand tones, but no sufficient production facilities are available.

Objective of the project:
The objective of this project is to install a manufacturing facility of microbial fertilizer. Products depicted in table-1 are proposed for manufacturing.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Name of the product</th>
<th>Classification</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nitrogen fixer Biofertilizer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Azotobacter chroococcum</td>
<td>Biofertilizer</td>
<td>N-fixer</td>
</tr>
<tr>
<td>B</td>
<td>Azotobacter vinelandii</td>
<td>Biofertilizer</td>
<td>N-fixer</td>
</tr>
<tr>
<td>C</td>
<td>Azotospirillum lipofelum</td>
<td>Biofertilizer</td>
<td>N-fixer</td>
</tr>
<tr>
<td>D</td>
<td>Rhizobium</td>
<td>Biofertilizer</td>
<td>N-fixer</td>
</tr>
</tbody>
</table>
The products in above table are biofertilizers which provides N, P of K to the plant.

**Details of technical ingredient (organisms) selected:**

1. **Nitrogen fixing organisms:**
   A. **Azotobacter** *(A. chroococcum, A. vinelandii, A. Lipoferum):*

   Azotobacter is a free-living bacterium that can fix atmospheric nitrogen into the soil, being a great source to obtain a natural biofertilizer that can be used in the cultivation of most crops. It is a great source of nitrogen to meet the needs of crops because also has the capabilities to cause a rejuvenation of soil microbiology to tap out the biological fixation of nitrogen.

   **Characteristics of Azotobacter:**

   The genus Azotobacter is comprised of bacteria that require the presence of oxygen to grow and reproduce, and which are inhabitants of the soil. There are six species of Azotobacter. The representative species is *Azotobacter vinelandii*.

   The bacteria are rod-shaped and stain negative in the Gram staining procedure. Some species are capable of directed movement, by means of a flagellum positioned at one end of the bacterium. Furthermore, some species produce pigments, which lend a yellow-green, red-violet, or brownish-black hue to the soil where they are located. Relative to other bacteria, *Azotobacter* is very large. A bacterium can be almost the same size as a yeast cell, which is a eucaryotic single-celled microorganism.

   *Azotobacter* has several features that allow it to survive in the sometimes harsh environment of the soil. The bacteria can round up and thicken their cell walls, to produce what is termed a cyst. A cyst is not dormant, like a...
spore, but does allow the bacterium to withstand conditions that would otherwise be harmful to an actively growing vegetative cell. When in a cyst form, *Azotobacter* is not capable of nitrogen fixation. The second environmentally adaptive feature of the bacterium is the large amounts of slime material that can be secreted to surround each bacterium. Slime naturally retains water. Thus, the bacterium is able to sequester water in the immediate vicinity.

A noteworthy feature of *Azotobacter* is the ability of the bacteria to "fix" atmospheric nitrogen, by the conversion of this elemental form to ammonia. Plants are able to utilize the ammonia as a nutrient. Furthermore, like the bacteria *Klebsiella pneumoniae* and *Rhizobium leguminosarum*, *Azotobacter vinelandii* is able to accomplish this chemical conversion when the bacteria are living free in the soil. In contrast to *Rhizobium leguminosarum*, however, *Azotobacter vinelandii* cannot exist in an association with plants.

*Azotobacter* can accomplish nitrogen fixation by using three different enzymes, which are termed nitrogenases. The enzyme diversity, and an extremely rapid metabolic rate (the highest of any known living organism) allow the bacterium to fix nitrogen when oxygen is present. The other nitrogen-fixing bacteria possess only a single species of nitrogenase, which needs near oxygen-free conditions in order to function. The enhanced versatility of *Azotobacter* makes the microbe attractive for agricultural purposes.

**Benefits of *Azotobacter***:

- It improves seed germination and plant growth
- *Azotobacter* is tolerant to high salts.
- It can benefit crops by nitrogen fixation, growth promoting substances, fungi static substances.
- *Azotobacter* is heaviest breathing organism and requires a large amount of organic carbon for its growth.
- It is poor competitor for nutrients in soil and hence its growth promoting substances, fungistatic substances.
- It thrives even in alkaline soils.
- *Azotobacter* is less effective in soils with poor organic matter content.
B. **Rhizobium** (*R. Japonicum)*:

*Rhizobium* is a genus of Gram-negative soil bacteria that fix nitrogen. *Rhizobium* forms an endosymbiotic nitrogen fixing association with roots of legumes. The bacteria colonize plant cells within root nodules; here the bacteria converts atmospheric nitrogen to ammonia and then provides organic nitrogenous compounds such as glutamine or ureides to the plant. The plant provides the bacteria organic compounds made by photosynthesis.

**Classification of Rhizobium**: The genus Rhizobium will consist of three reorganized species: *R. leguminosarum*, which will contain three biovars (biovar trifolii, biovar phaseoli, and biovar viciae); *R. meliloti*; and *R. loti*. The reorganization combines into one the former species of *R. leguminosarum*, *R. trifolii*, and *R. phaseoli*. The fast-growing members of the cowpea rhizobia and the former species *R. lupinus* have been included in the species *R. loti*. The new genus, *Bradyrhizobium*, is made up of one species, *B. japonicum*, which consists of the former species *R. japonicum*, plus the slow-growing members of the cowpea rhizobia. The newly proposed classification of *Rhizobium* is as follows.

**GENUS I**: *Rhizobium R. leguminosarum* biovar trifolii biovar phaseoli biovar viciae *R. meliloti* *R. loti*-fast-growing, sub-polar flagellated strains from Lotus and Lupinus with strong affinity for *L. corniculatus*, *L. densiflorus*, and *Anthyllis vulneraria* (but also nodulates *Ornithopus sativum*). Includes the fast-growing strains nodulating Cicer, Sesbania, Leucaena, Mimosa, and Lablab.

**GENUS II**: *Bradyrhizobium* Slow-growing, polar or sub-polar flagellated strains nodulating soybean, Lotus uliginosus, *L. pendutulatus*, and Vigna. Includes those slow-growing strains nodulating Cicer, Sesbania, Leucaena, Mimosa, Lablab, and Acacia. The possibility exists that other species will eventually be defined within this genus, but for the present it is suggested that, other than *B. japonicum* (the type species), the various cultures be designated ex. *Bradyrhizobium sp.* (Vigna), *Bradyrhizobium sp.* (Cicer), etc.

Recently, two more genera have been added to the family Rhizobiaceae. They are *Sinorhizobium* and *Azorhizobium*, nodulating soybean and Sesbania, respectively. The combined results of both somatic and flagellar reactions have served to distinguish strains within a cross-inoculation group. Serological methods can be used as a means of
obtaining information on the distribution of strains that can be recognised within an area, on widely separated areas, on the plant or within a nodule. Serologically, it is known that a single nodule contains a homogeneous population of a single strain of *Rhizobium*, although it is not uncommon to find more than one strain on the same plant.

C. *Acetobacter xylinum*:

*Acetobacter* bacteria, such as *Acetobacter diazotrophicus* that can be isolated from coffee plants or sugarcane, are acid-producing, nitrogen-fixing bacteria. In fact, the *A. diazotrophicus*-sugarcane relationship, first observed in Brazil, was the first report of a beneficial symbiotic relationship between grasses and bacteria through nitrogen fixation. Nitrogen-fixing bacteria are important in modern agriculture - exploiting these bacteria would decrease the present dependency on nitrogen fertilizers, which would have positive results for the ecosystem and the health of humans and other animals. Other strains can be found in samples from Japanese rice vinegar (komesu) or unpolished rice vinegar (kurosu).

*Acetobacter* is an obligatory aerobic, nitrogen-fixing bacteria that is known for producing acid as a result of metabolic processes. While all nitrogen-fixing bacteria contain nitrogenase in order to utilize atmospheric nitrogen gas as a source for metabolic biosynthesis, different nitrogen-fixing microorganisms protect the oxygen-sensitive microorganisms from oxygen exposure in different ways. *A. diazotrophicus* has been called interesting because it carries out nitrogen fixation under aerobic conditions. It needs oxygen for the production of large quantities of ATP required for nitrogen fixation; however, little is known about the mechanism or system that protects the nitrogenase under aerobic conditions. *A. diazotrophicus* is a plant endophyte and has been said to be capable of excreting about half of its fixed nitrogen in a form that plants can use.

*Acetobacter* bacteria can be found in symbiotic relationships with many different plants, such as sugarcane and coffee plants, as well as in fermenting vinegar. Endophytes are prokaryotes that associate with plants by colonizing their internal tissues. Many of these bacteria have been observed to promote plant growth, but the mechanisms behind this relationship are not yet fully understood. Below is a table of places where *A. diazotrophicus* can be found.

**Table-2**
Table from Muthukumarasamy et al. 2002

<table>
<thead>
<tr>
<th>Plant</th>
<th>Tissue/Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane</td>
<td>root, root hair, stem, leaf</td>
</tr>
<tr>
<td>Cameroon grass</td>
<td>root, stem</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>root, stem tuber</td>
</tr>
<tr>
<td>Coffee</td>
<td>root, rhizosphere, stem</td>
</tr>
<tr>
<td>Ragi</td>
<td>root, rhizosphere, stem</td>
</tr>
<tr>
<td>Tea</td>
<td>root</td>
</tr>
<tr>
<td>Pineapple</td>
<td>fruit</td>
</tr>
<tr>
<td>Mango</td>
<td>fruit</td>
</tr>
<tr>
<td>Banana</td>
<td>rhizosphere</td>
</tr>
<tr>
<td>Others</td>
<td>mealy bugs, VAM spores</td>
</tr>
<tr>
<td>Others</td>
<td>internal environment</td>
</tr>
</tbody>
</table>

2. Phosphate solubilizing microorganisms:
Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2% of plant dry weight. Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca²⁺, Mg²⁺, Fe³⁺ and Al³⁺, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. As the results, the amount available to plants is usually a small proportion of this total.

Several scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate.

Mechanisms of phosphate solubilization: The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms. Production of organic acids results in acidification of the microbial cell and its surroundings. The production of organic acids by phosphate solubilizing bacteria has been well documented. Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. Also, 2-ketogluconic acid
is another organic acid identified in strains with phosphate solubilizing ability. Strains of *Bacillus* were found to produce mixtures of lactic, isovaleric, isobutyric and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers

A. **Bacillus megatherium:**
*Bacillus megatherium* is phosphate solubilizer. It is a rod-shaped, Gram-positive, endospore forming, species of bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillus form. *Bacillus megatherium* is a rod shaped bacteria and one of the largest eubacteria found in soil. Groups of the bacteria are often found in chains where the cells are joined together by polysaccharides on the cell walls. *Bacillus megatherium* is able to survive in some extreme conditions such as desert environments due to the spores it forms. Where there are favourable conditions the spores can survive.

A. **Pseudomonas putida:**
*Pseudomonas putida* is a phosphate solubilizer. It is gram-negative rod-shaped saprotrophic soil bacterium. Based on 16S rRNA analysis, *P. putida* has been placed in the *P. putida* group, to which it lends its name.

3. **Potash Mobilizers:**
This bacterium help to mobilize the insoluble form of potassium for crop growth at a faster rate. Seventy percent of insoluble potassium is made available to the crop plants within 25 days of bio-potash application in soil. Reduces cost of potash application by 50-60%.
- Improves resistance of crop plants
- Resistant to a wide range of soil pH and temperature.
- Suitable to apply for all crops.
- Improves crop growth and yield by 20-30%
- Compatible with other bio-fertilizers

A. **Frateuria Aurentia:**
The best example of potash mobilizer is *Frateuria Aurentia*. It is a bacterium and useful in plant nutrition.
C. MASS PRODUCTION AND QUALITY CONTROL

1. Mass production of Biofertilizers:

**Media Preparation and Starter Culture**

Bacteria require different nutrients for their growth. These include: a) organic carbons source, b) nitrogen source and c) a variety of other elements dissolved in water. Blue Green Algae (BGA), that can fix atmospheric carbon dioxide, does not require any carbon source and the nitrogen-fixing bacteria, which can fix atmospheric nitrogen, do not require any nitrogen source. A medium is an aquatic solution of a variety of organic and inorganic compounds that can supplement the above requirements for the growth of different microorganisms. Generally, media are of two types: a) General media and b) Specific media. General media is constituted for the growth of most of the microorganisms. Such a medium contains all the ingredients required for the growth of any microorganism. A specific medium is constituted for the growth of specific or specific group of microorganisms. During constitution of such medium, one or few additional components are added and /or one or few components are deleted from the general media depending upon the requirement of the specific microorganism. Again, according to the physical appearance, media are of two types: a) Liquid media and b) Solid media. The liquid medium is solidified by the addition of solidifying agent- agar-agar. Liquid medium can harbor bacterial growth suspended in the media, whereas solid medium harbors microbial growth on the surface. Solid media may be prepared as slant or plate.

**Sterilization of medium in autoclave:** The media is then autoclaved at 121°C temperature and 15LB pressure. The process is as follows

- Check the water level of the autoclave to note whether the heating coil is completely immersed in water, add water, if necessary.
- Put the conical flasks, petridishes and the beaker with test tubes inside the basket of the autoclave.
- Set the lid, tie the screws of the autoclave and switch on the power supply.
- Keep the outlet of the steam open and wait until the inside air is completely released from the autoclave and only steam is coming out.
- When the inside air is completely released close the outlet and observe the meter indicating the inside pressure.
- When the pressure rises to 15 lbs, adjust the regulating valve to keep the pressure constant.
- Keep this pressure for 30 min and then close the regulating valve and switch off the power supply.
- When the pressure comes down to zero, open the regulatory valve to release the steam and then open the lid and take out the contents.

**Prepare slants and plates:** The slants are necessary for culture storage. The slant preparation process is as follows-

- Put the test tubes containing medium in slanting position on the table with the help of a wooden blade and allow cooling down. The medium will be solid after cooling down and thus slants are prepared.
- Take the conical flask containing molten media with agar agar and the petridishes in the laminar flow cabinet and allow the medium to cool down to 50°C.
- Open each petridish by slightly lifting the upper lid, pour 15-20 ml of medium and close the lid.
- Keep to cool down and solidify and thus plates are prepared.

**Preparation of Starter Culture:**
- The starter culture is a little amount of bacterial suspension, which is added to the medium to start the growth of that bacterium. Twin flask is a pair of flasks of identical size joined together by a latex tube. For the preparation of starter culture, this type of flask is used. Each flask contains a side arm below the neck position. The latex tube joining the two flasks is held together by this glass tube. The benefit of the use of the twin flask is that contamination can be avoided.

**Fermentation:**

A fermenter is a device in which the optimum conditions for the microbial growth and activity is established artificially. This device may be used for the production of microbial metabolites such as antibiotics or enzymes; it may also be used for the growth of microorganisms i.e. production of microorganism itself.
A low cost production unit has been developed for the production of microbial inoculants to be used as biofertilizers such as *Rhizobium*, *Azotobacter*, and Phosphate Solubilising Bacteria. This device can be prepared by investing small amount of money as compared to the scientific fermenter used in laboratories for research purposes.

**Sterilization of the fermenter:**

Fermenter is a metallic vessel for moist sterilization of any article. The principle of moist sterilization lies in the fact that when water is boiled in a closed system, the water vapor produced due to boiling accumulates within the vessel and increases the inside pressure. Thus the boiling point of water increases beyond 100°C, which is the boiling point of water in normal atmospheric pressure. In this condition, the steam, released from the boiling water is of higher temperature. If any article placed in this vessel in such condition, the high temperature destroys the microorganisms present in or on the article.

**Inoculation, Growth, Quality Testing and Termination of growth:**

Inoculation means addition of starter culture to the medium in the fermenter. For the production of microbial biofertilizers a small amount of suspension of the desired bacterium in pure form is inoculated to the medium. Care should be taken to maintain the quality of starter culture, as extent of purity (no contaminants should be allowed), size of the starter culture (in terms of culture volume and density of cell) and stage of growth. Greater the size of starter culture, lesser the chance of contamination. If the starter culture is inoculated in its log phase, rapid initial growth will occur. Maintenance of proper physical and chemical environment inside the fermenter is essential for proper growth of microorganism. Quality testing, in this case, is enumeration of cell density and its purity in the broth. When the cell density reaches the desired level, growth is terminated and the culture is ready for mixing with carrier. The time period required for optimum cell density is thus standardized.

**Carrier Preparation**

Carrier is a medium, which can carry the microorganisms in sufficient quantities and keep them viable under specified conditions and easy to supply to the farmers. A good carrier should have the following qualities:

- Highly absorptive (water holding capacity) and easy to process.
- Non- toxic to microorganisms.
- Easy to sterilize effectively.
- Available in adequate amount and low-cost.
- Provide good adhesion to seed.
- Has good buffering capacity.

Different carriers are available in the market like, Charcoal, Peat, Lignite, Rice husk etc. But considering all the above qualities Azolla powder is the most suitable carrier in this region. This is due to-
- It has high water holding capacity (360%).
- It has good pH buffering capacity.
- It contains nutrient so bacteria can remain viable for a long period.
- It is easily available in this region.

**Formulation:**
- Inoculation of the carrier with the culture broth means the mixing of broth and carrier. This operation must be done in aseptic conditions to avoid any contamination.

**Quality control of formulation:**
- The quality of the carrier-based inoculum depends upon the viable cell count and the presence or absence of contaminants. A good culture must contain about $10^7 - 10^8$ viable cells or CFUs per g of culture. No contaminants are permissible at $10^{-5} - 10^{-6}$ dilutions. These critical values differ according to the type of biofertilizer. As per I.S.I., in case of *Rhizobium*, the carrier based culture must contain at least $10^7$ cell or CFUs per g of culture and no contaminants are permissible below $10^{-6}$ dilution. In case of *Azotobacter*, So, the enumeration of cells density and contaminants are important task in the production of carrier based microbial biofertilizer.

**Bioactivity of Azotobacter:**

Nitrogenase is the enzyme catalyzing the reduction of nitrogen into ammonia. This enzyme can also reduce acetylene into ethylene as well. Acetylene and ethylene can easily be measured by a gas chromatograph. In a closed system, if a portion of gas is substituted by acetylene and acetylene is allowed to be reduced for a certain period, the proportion of acetylene and ethylene can be
measured by passing the mixture of gas through the column of gas chromatograph and measuring the peak developed.

The nitrogenase activity (hence, the nitrogen fixation activity) of Azotobacter or other free living bacteria can be extrapolated by Acetylene Reduction Assay (ARA) method, but this technique can not be applied in case of Rhizobium as this bacteria can not fix nitrogen in free condition. In this case the plant containing the nodules is to be taken for assay.

**Bioefficacy of Rhizobium (Nodulation):**
The study of nodulation efficiency of Rhizobium is an important task in Biofertilizer Technology. The number of viable cells in the inoculum can easily be enumerated by dilution plating method but this technique will not show whether the viable cells have retained their nodulation efficiency during the long term laboratory procedure like preservation, production and storage. This technique will enumerate the number of viable cells, which have retained the nodulation capability, this is well known that this nitrogen-fixing bacteria can not fix nitrogen unless it is symbiotically harbored in the nodule of leguminous plant.

**Application of Microbial Biofertilizer:**
Application of the microbial biofertilizer is an important step in the Biofertilizer Technology. If the microbial inoculant is not applied properly, the benefits from the biofertilizer may not be obtained. During application one should always remember that the most of the microbial biofertilizers are heterotrophic, i.e. they can not prepare their won food and depend upon the organic carbon of soil for their energy requirement and growth. So, they either colonise in rhizosphere zone or live symbiotically within the root of higher plants. The bacteria which are colonised in the rhizosphere zone obtain their organic carbon compounds from the root exudes of the higher plants. The symbiotic ones obtain organic carbon directly from the root. So, microbial inoculants must be applied in such a way that the bacteria will be adhered with the root surface. So, in case of transplanting crops, the inoculant are applied through roots, and in case of the crops in which seeds are sown directly in the field, the inoculants are applied through the seeds so that they can colonize in the rhizosphere region when the young roots are emerged after germination of seed.

On the basis of the above principal, the following inoculation methods has been developed:

1. Inoculation of the seeds by slurry inoculating technique
2. Inoculation of seeds by seed pelleting technique
3. Inoculation of the seedlings
4. Inoculation of the soil by solid inoculation technique
5. Inoculation of soil by liquid inoculation technique
Laboratory setting and operations

1. Aseptic techniques:
Working in absence of contaminants is very important thing. Aseptic technique is essential for all pathology work and must be thoroughly practiced and mastered.

Insect pathogenic fungi or bacterial cultures for insect pathology must be pure. This means that they must be free of any living microbes other than the one required. The presence of unwanted microorganism (fungi or bacteria) is known as CONTAMINATION and the microbes responsible for contamination are referred to as CONTAMINANTS. We use aseptic technique so that we can handle, or manipulate microorganisms without appearance of contaminants into the culture. Aseptic technique also helps to protect the operator from potential infection from pathogenic organisms.

ALWAYS use aseptic technique when handling microorganisms and also when preparing microbiological media in which to grow these organisms.

Sterilization:
Elimination of all viable microbes from a material is known as sterilization. Sterilization is nonselective process. It is very important stage for any microbiological work. The success of proper sterilization ensures quality of final product. ALL equipment and media to be used during the handling of the microorganism must be sterile.

Disinfection:
Disinfection is a way to reduce the contaminant load. It removes potentially infective microbes, but does not render the object sterile.

Many different methods of sterilization are being used. The sterilization method you use depends on the equipment you have and what it is you are sterilizing. As a general rule, the following methods are most appropriate.

Microbial growth media
Wet heat sterilization, usually using an autoclave although a domestic pressure cooker will do just as well
1. Raise the temperature to 121°C and the pressure in the closed chamber to 15 psi for 15-20 minutes.
2. DO NOT over fill vessels containing liquid; leave a large space at the top of all bottles to allow for expansion and boiling of the liquid on heating in the autoclave.
3. Loosen screw caps before autoclaving.

**Laboratory growth media**
Sterilize as above using wet heat sterilization or dry heat sterilization in an oven (see lab techniques) at 160°C for 1-2 hours. If you have no autoclave, pressure cooker or oven, you can use certain chemical agents such as strong acids or alkalines, phenols or ethylene oxide. All chemical methods are potentially hazardous to the operator and should be avoided where possible. Methods for chemical sterilization can be found in the Plant Pathologists Pocketbook (see Appendix II).

**Small pieces of equipments:**
Sterilize glass rods and metal tools by dipping them in 70% ethanol (alcohol) and then flaming to burn off the alcohol. Sterilize inoculating loops and needles by holding in a flame until red-hot.

**Laboratory benches**
Swab the working surface with 70% alcohol or chemical disinfectant to prevent the introduction of contaminants. NEVER allow anything, which is sterile to come into DIRECT contact with the bench.

**Other methods of sterilization are available.**
1. Ultraviolet radiation: can be useful for benches and clean rooms.
2. Gamma radiation: is used for the sterilization of plastics in industry.
3. Filtration: using filters of a maximum pore size of 1nm, generally used to sterilize small quantities of liquids which are unstable at high temperatures. However these methods are not often used in insect pathology.

**Use aseptic technique during all microbial transfer**
Make sure that ALL equipment used is properly sterilized using the most appropriate method.
2. Sterilization techniques

Metal
Sterilize needles, wire loops etc. by heating them in a flame until red-hot.

Glassware
Can be sterilized in a hot air oven, a domestic oven will do. Do not pack glassware too tightly.

Media
Wet heat sterilize in an autoclave or pressure cooker for forty (40) minutes.

DRY HEAT OR OVEN STERILIZATION
Using the oven method of sterilization, glassware will be sterile as follows:

Oven temperatures and time for sterilization
- 120°C 8 hours
- 140°C 3 hours
- 160°C 1 hour
- 180°C 20 min.

Autoclave or pressure cooker:
You can use an autoclave or pressure cooker at 15 psi for 15 minutes, this is the standard recommended for most microbiological media. Under certain circumstances, you may be required to alter the temperature/pressure for sterilization. The table below gives the temperatures which will be achieved at various pressures in pounds per square inch (psi), the time required for sterilization should be stated in the instructions given:

Autoclave pressures and temperatures:
- 5 psi 107°C
- 7 psi 110°C
- 10 psi 115°C
- 15 psi 121°C
- 20 psi 126°C

POINTS TO REMEMBER WHEN USING A PRESSURE COOKER OR AUTOCLAVE
- Use distilled water (if available) in pressure cookers and autoclaves.
- Ensure that all bottles and containers are heatproof.
- Never fill bottles and containers to the top, always leave a gap between the liquid and the lid to allow the liquid to expand and boil.
Loosen the caps of all bottles and containers before placing in a pressure cooker or autoclave, this allows the steam to enter the containers and sterilise the contents.

Always read the manufacturers instructions before using a pressure cooker or autoclave (the instructions for using pressure cookers and autoclaves below are only meant as guidelines).

Using the pressure cooker:
1. Put a little water in the bottom of the pressure cooker.
2. Put the media in heatproof containers or in sterilisable plastic bags.
3. Put the containers in the pressure cooker on the trivet* and make sure that the lids of bottles are loose.
4. Close the pressure cooker but do not put the weight on top on the steam valve. Put the pressure cooker on the heat.
5. Once the water in the pan starts to boil, steam will come out of the open valve. Allow steam to pass out of the valve for about 5 minutes, and then put the weight on top of the valve.
6. The steam builds up in the inside of the pressure cooker until it reaches the correct pressure. The steam then lifts the weight and starts to escape. The weight acts to regulate the pressure inside the pan.
7. As soon as the steam starts to escape, time the sterilization from the point at which the steam starts to escape. Turn down the heat during sterilization so that the steam is only just escaping. NOT rushing out.
8. Turn off the heat after the recommended sterilization time.
9. Leave to cool before removing the weight (or opening the valve).
10. Wait until pressure is completely reduced then lift the weight off the valve. Any remaining steam will escape and the pressure cooker is then safe to open.

N.B. Never open a pressure cooker or autoclave until the valve has been opened to release the pressure.

USING A MANUAL AUTOCLAVE
1. Put in sufficient water.
2. Load the articles to be sterilised into the autoclave.
3. Screw down the lid.
4. Open the steam valve.
5. Switch on. If there are high and low switches on the autoclave make sure both are switched on.
6. Let steam come out for at least five (5) minutes before closing steam valve. Continue heating until the pressure is up to 15 psi.
7. Adjust pressure and turn the heat down or the high switch off.
8. Leave to steam for the appropriate time then turn off the autoclave.
9. Leave to cool to reduce the pressure to zero.
10. Open the steam valve to release any remaining pressure.
11. Wait five (5) minutes before opening the lid.

Using an automatic autoclave:
1. Open the autoclave and fill with water to the right level.
2. Set the timer and switch on the heat.
3. Set the "power" switch to the "off" position as soon as the alarm sounds, or light flashes.
4. Wait until the pressure falls and the temperature reaches 80°C or less.
5. Open the autoclave.

3. Agar

Agar is used to solidify nutrient media for growing bacteria and fungi, as it becomes liquid at 100°C and sets at 40°C. Agar can be obtained either as pure agar powder for adding to nutrient solutions which are prepared in the laboratory or ready mixed as a nutritive substrate in powder form with nutrients added according to specific recipes. Sabouraud dextrose agar (SDA) and Malt extract agar (MEA) can be bought ready prepared or can easily be made up in the laboratory using pure agar powder plus the raw ingredients.

N.B. Ready mixed agar products are generally more expensive than purchasing the pure agar powder and the media components separately.

Agar powder will only dissolve in boiling water, once dissolved, the solution will remain liquid until it has cooled to 40°C. It will then solidify into a firm gel. If required, agar can be reheated by steaming or autoclaving and will become liquid again at 100°C. Agar can be used in Petri dishes (plates) or in bottles (slopes). If agar is to be used in Petri dishes, it should be sterilised in a large bottle/bottles and distributed after sterilisation. If it is to be used for making agar slopes, it should be distributed into the small
bottles once it has dissolved and sterilised in the individual bottles. Several different recipes based on agar are given below:

**Tap water agar (TWA):**
1. Put 15 g pure agar and one (1) litre of tap water into a conical flask.
2. Boil the agar in the water until dissolved.
3. Sterilize at 15 psi for 20 minutes.

**Potato carrot agar (PCA):**
This is a very weak medium used for storing cultures and can be made with antibiotic solution added for making isolations from insects.
1. Grate 20 g potato and 20 g carrot (wash the potatoes and carrots well before grating).
2. Boil grated potato and carrot in one (1) litre tap water for one (1) hour.
3. Strain through a fine sieve - DO NOT press.
4. Add 20 g agar to the strained liquid.
5. Heat the solution until the agar is dissolved, then add water to make up to one (1) litre.
6. Sterilize at 15 psi for 15 minutes.

N.B. Antibiotic solution may be added to PCA for use in isolations from insects or cleaning up cultures after bacterial contamination.

**Antibiotic solution:**
1. Weigh out 0.05 g of Chloramphenicol powder into a clean, dry Universal bottle.
2. Add 10 ml of 90 - 95% alcohol (ethanol). Do not sterilise this solution.
3. Add antibiotic solution to the sterilised agar at the rate of 1ml in 100 ml agar medium (i.e. 10 ml in 1 litre).
4. Gently invert the bottle several times to distribute the solution evenly throughout the medium, but don’t shake the bottle as this will create air bubbles.
5. Now autoclave the medium again for just 10 min at 10 psi and 115°C (this can be done in a pressure cooker using the small part of the weight only) to ensure total sterility.

N.B. Wear gloves at all times when handling concentrated antibiotics. Chloramphenicol is poisonous.
**Malt extract agar (MEA):**
1. Boil 20 g malt extract in one (1) litre water until dissolved.
2. Add 20 g agar.
3. Boil until agar is dissolved.
4. Sterilize at 15 psi for 15 minutes.

**Potato dextrose agar (PDA):**
You will need:
- Potato 200g
- Dextrose 20 g
- Agar 20 g
- Tap water 1 l

1. Take 200g potatoes.
2. Scrub the potatoes clean - DO NOT PEEL.
3. Cut into 12 mm cubes.
4. Weigh out 200g potatoes.
5. Rinse rapidly in running water.
6. Place in one (1) litre water.
7. Boil until soft (1 hour).
8. Mash and squeeze as much of the pulp as possible through a fine sieve.
9. Add 20 g agar and boil till dissolved.
10. Add 20 g dextrose and stir till dissolved.
11. Make up to one (1) litre with water.
12. Sterilize at 15 psi for 20 minutes.

**Sabouraud dextrose agar (SDA):**
You will need:
- D-glucose (Dextrose or Maltose) 200 g
- Peptone 20 g
- Agar 20 g
- Tap water 1 l

1. Dissolve the D-glucose, peptone and agar in the tap water by boiling them together.
2. Autoclave at 115 psi for 15-20 minutes.
**Ready mixed powdered agar products:**
Follow the instructions given by the manufacturer.

**Pouring agar plates:**
1. Autoclave the agar (to melt and sterilize). Cool the agar until hand hot.
2. Lay out newly opened plastic Petri dishes OR sterile glass Petri dishes in a clean area.
3. Pour the agar into the dishes to a depth of 0.5 cm (approximately 15 ml in a 9 cm Petri plate)
4. Allow to cool uncovered in a sterile air cabinet, keeping the lids within the sterile airflow (do not touch the inside surface of the lids as this will cause contamination).

N.B. If you do not have a sterile air flow cabinet, replace the lid of each Petri plate immediately after adding the agar and allow the agar to set. Once cool, any condensation, which has collected on the lids of the agar plates can be removed by taking the lid and giving it a short sharp shake. Replace the lids immediately.
5. Cover with lids.
6. If not needed immediately, store in the refrigerator (5°C) for several weeks.

**Preparing slopes in bottles:**
Use 25-30 ml Universal bottles; use SDA for *Metarhizium* and PDA, SDA or MEA for *Beauveria*
1. Prepare agar.
2. Put approximately 7 ml of agar in each Universal bottle.
3. Place the bottles in the autoclave with lids loose and autoclave as instructed in the agar recipe.
4. Tilt the bottles so that the agar forms a slope inside the bottle and let them cool.
5. Tighten the lids and store in a refrigerator until use. For small-scale production of spores use 300ml medical flats or 700 ml square sided whisky bottle.

1. Prepare agar.
2. Put 100 ml of agar in each 700 ml bottle and 40ml agar in each 300 ml bottle.
3. Autoclave the bottles (with lids loose) as instructed in the agar recipe.
4. Tilt the bottles and let them cool.
5. Tighten the lids and store in a refrigerator until use

4. Safety in the laboratory:
   • NO SMOKING
   • NO EATING
   • NO DRINKING
   • GOOD WASHING FACILITIES
   • GOOD DISPOSAL FACILITIES
   • RESPONSIBILITIES

The laboratory supervisor/manager must ensure that:
1. The laboratory is a safe working environment.
2. Equipment is safe.
3. All technicians are aware of the dangers.
4. All technicians are properly trained to do their work.
5. Be responsible for chemicals.

Technicians are responsible for:
1. Keeping benches clean and uncluttered.
2. Keeping floors clean.
3. Cleaning and proper storage of glassware.
4. Storing chemicals in proper places (cupboards, stores).
5. Maintaining equipment.
6. Bringing any problems or potential problems to the attention of the supervisor.

Clothing:
Technicians must always wear the right kind of protective clothing.
• Laboratory coats should be worn (if the room temperature is high this may prove to be impractical).
• Facemasks must be worn when dealing with dusts.
• Gloves must be worn when handling chemicals.

Chemicals:
ALWAYS read the label on the container.
1. Orange background with black cross: harmful.
2. Orange background with black skull and crossbones: toxic.
3. Orange background with black fire: flammable.
4. Orange background with black Bar or hand being eaten away: corrosive.
5. There may be written warnings, but they are often only in one language.

Harmful solid! Target organ:
Nerves! Possible teratogen*!

ALWAYS keep a list of chemicals and the dangers associated with them.
NEVER store chemicals in anything other than their original container, with the correct label.
ALWAYS keep flammable and toxic chemicals in a securely locked cupboard.

Biological hazards:
The entomopathogenic fungi used for locust control are very safe, BUT they may cause allergic reactions if they are inhaled. Some common contaminants e.g. Aspergillus spp. are harmful. Technicians must learn to recognise and dispose of contaminants safely.
ALWAYS keep benches, equipment and protective clothing clean.

First aid:
ALWAYS keep a first aid box in a secure place, in full view and check it regularly. Write all injuries in an accident book which should be kept next to the first aid box It is ESSENTIAL that at least one person in a laboratory is trained in first aid procedures.
KEEP AN ANTIDOTE FOR ANY ESPECIALLY DANGEROUS CHEMICALS
TYPE OF EQUIPMENT IN THE LABORATORY

Introduction

When considering the setting up of a laboratory for fermentation, a number of points should be kept in mind.

Scale of operation.

Clearly, at the bench-top scale (up to 3 litres volume), little additional adaptation would be required in a standard microbiology laboratory. Larger-volume fermenters (usually mounted on skids, trolleys, or frames) require more extensive preplanning of the operational area. Such fermenters, room 5-50 litres working volume, might well be termed research scale; anything larger would come into category of a small pilot plant. Small scale processing is from 50 to several hundred litres working volume.

Type of fermenters.

Type of reactor, used at every operational scale, has a much higher degree of operational flexibility than most other fermenters.

Number of fermenters.

The use of one or two bench fermenters will require only a source of electric power, water, and access to a small autoclave. Operation of a number of large vessels will have considerable impact on lab design; for example, it becomes worthwhile arranging steam line to each fermenter point to allow for in situ sterilization.

Nature of fermentation process.

This really relates to the type of organism to be used; the use of fungi will require equipment quite different in some respects from that employed for "traditional" microbial fermentation.

In the description of a fermentation laboratory, which follows, it should be remembered that this describes the "ideal", a lab constructed a finite for the
purposes of microbial fermentation. Such an "ideal" is rarely achieved, but is always a useful target.

**Pilot Plant**

**General Description**

A fermentation laboratory is primarily a microbiology laboratory incorporating advanced technology equipment, and as such should be designed to maximize efficiency while maintaining a high safety standard. The general layout of the laboratory will depend on the shape and size of the room allocated, and it is advisable for laboratory personnel and scale production.

The laboratory is divided into two or three distinct areas:

(i) **Wet floor area:** This houses all fermenters, stills, autoclaves, and centrifuge.

(ii) **General laboratory area:** Used for medium preparation and basic "wet" processes, i.e. dry weights, viscosity, pH, and measurements

(iii) **Formulation area.**

**Floor Areas**

A fermentation laboratory has two distinct areas, the wet floor and the dry floor.

**Wet Floor**

All fermenters, including bench top varieties, should be situated on the wet floor area so that spillages, intentional or not, can do no damage either in the laboratory or to people and equipment situated on the floor below. Wet floor should be constructed to meet two main criteria, it should be non-slip, whether wet or dry, and should be easily cleaned. These factors are often not compatible, i.e. the rough surface required to make the floor non-slip is often ideal for harbouring dirt. The wet floor should be constructed so that it slants towards a central drainage channel (fermenters should be leveled using adjustable feet or blocks); the channel itself should slant towards the drainage output of the laboratory, and must be covered by a stainless steel or heavy-duty plastic grid to prevent accidents.

**Dry Floor**
The dry floor area of the laboratory should be covered with seamless sheet vinyl, which has a smooth surface and does not catch dirt.

The junction between the wet and dry floor areas of the laboratory is an area of potential danger, as sheet vinyl is slippy when wet. It is advisable to introduce an area where shoes can be dried between the two floor types.

Routine cleaning of both floor areas is essential. A wide range of detergents/disinfectant is available, and the product chosen will depend on the biocidal effectiveness required. Because of the nature of equipment and fermenter contents, trained personnel only should clean the wet floor.

**Laboratory Equipment**

**Shake Flasks and Bottles**

A - Standard shake flask or the Erlenmeyer flask
B - "Flying saucer" shake flask
C - Shake flask with baffles
D - Flat bed "Thompson" or "Roux" bottle.

These pieces of glassware can vary in size and form and in some instances have been designed and developed for specialist application.
Shaker Tables

Rotary shaker is a platform with many clamps to fit conical flasks. A motor below it shakes the platform.

This shaker is used for the following purposes:

- To make a suspension of soil or bacteria.
- To aerate the liquid bacterial culture.

To prepare a solution which requires shaking for a prolonged period.

Shaker tables were designed to assist with oxygen transfer. These tables are designed to run for long periods of time and be free from vibration. The tables are driven by a motor, and normally a rotary shaking action or reciprocating shaking action is produced.

These shakers have to be robust and reliable with no vibration and silent running conditions. One can have a more sophisticated shaker by having an incubator shaking cabinet for shake-flask fermentation in a precisely defined environment. These cabinets can control the temperature, illumination, gaseous levels, and humidity.

Increasing the speed of a shaker can increase the oxygen transfer rate of a particular flask, therefore the optimum speed for that flask and culture has to be found by trial and error.

Shake Flask Volume

The lower the volume of medium in a shake flask, the better will be the OTR (Oxygen Transport Rate). The minimum volume that can be practically obtained
(e.g. 50 ml in a 250 ml shake flask) should give the best OTR and hence the best results. This will also be dependent on sample volume. Very low volumes can only be used for short-term fermentations, otherwise the medium will evaporate and the nutrients would become too concentrated for the culture to perform satisfactorily.

**Common glassware:**

All the articles used in the laboratory for the measuring of liquid, preparation of solution, etc. are made up of glass. This is because glass is less reactive. The common glasswares are as follows:

1. **Pipette:**
   A pipette is a graduated tube with a pointed end. Liquid of a particular volume can be taken in a pipette by sucking and liquid of desired volume may be released through the pointed tip. Pipette may be of different volumes: 1 mL, 5 mL, 10 mL and 25 mL.
How to use a pipette:

In the figure, there is a 10 mL pipette; say, you have to pipette out 6 ml of a solution 'x', then,

i) Dip the pointed tip ‘C’ of the pipette in the solution ‘x’.

ii) Suck the solution ‘x’ through the end ‘A’ of the pipette.

iii) After sucking, place the right index finger on the end ‘A’ so that the sucked solution (x) remains in the pipette. Slowly release the finger, if ‘x’ has been sucked above the mark ‘0’ of the pipette, until ‘x’ reaches the ‘0’ level mark.

iv) Place the tip ‘C’ of the pipette on the glassware into which the 6 ml of ‘x’ solution is to be taken.

v) Slowly release the finger as before until ‘x’ reaches the mark ‘6’ of the pipette.

vi) Similarly, you may take solution of any volume from 1 mL to 10 mL, using this pipette.

Measuring Cylinder:

Measuring cylinder is a graduated cylinder used to measure the volume of a liquid. While the pipette is used to measure liquid of less volume, measuring cylinder is used to measure liquid of greater volumes like 25 mL, 50 mL, 100 mL, 500 mL, 1000 mL etc., and accordingly, measuring cylinders of different volumes are available.
The figure shows a measuring cylinder of 1000 mL capacity. Care should be taken while observing the volume of a liquid, the level of the liquid and your eye must be in the same plane.

**Volumetric flasks:**

Volumetric flasks are used to prepare standard solution of a known concentration. It has a narrow neck with an indication mark denoting the exact level of the solvent to be taken to make up the required volume. It also has glass stopper at the mouth. The flask may be of different volumes, 25 mL, 50 mL, 100 mL, 250 mL or 1000 mL.
How to use volumetric flask:

If you have to prepare 100 ml 5% NaCl solution, take 5 g NaCl within the flask. Add 50 -70 ml distilled water and close the stopper. Shake the content gently until the salt is dissolved completely. Then adjust the volume up to the mark by slowly adding distilled water.

Beaker:

Beaker is a flat-based cylindrical glassware required for the preparation of a solution. Beakers may also be of different volumes viz., 50 mL, 100 mL, 500 mL or 1000 mL

Conical flasks:
As the name indicates, the flask is conical in shape with flat base and gradually narrowing upper part. Conical flask of different volumes are available, 100 mL, 150 mL, 250 mL, 500 mL, 1000 mL, 1.5L, 3L etc. These flasks are used for the culture of plant tissue, microorganisms and also in the preparation of culture media.

2. Culture tubes and petridishes:
Both petridishes and culture tubes are required for culturing plant tissue and microorganisms. Solid medium for plant tissue culture can be prepared as slant within a culture tube. Culture tubes may be of different sizes and volumes. Slants can also be used for culturing microorganisms, especially for germplasm conservation. Petridishes are used for the preparation of solid medium, plates, to develop colonies of microorganisms. A colony is a group of large number of cells developed from a single cell on the surface of solid medium.

Burette:
Burette is a cylinder of about 50 mL volume. Its one side is open and the other side is pointed to which a stopcock is fitted. It is fitted to a stand by a clamp. It is used during titration.

Burettes, such as this one, read from the top down.
1. **Autoclave:**

An autoclave is a device for moist sterilization of any article. The principle of moist sterilization lies in the fact that when water is boiled in a closed system, the water vapour produced due to boiling accumulates within the system and increases the inside pressure. Thus the boiling point of water increases beyond 100°C, which is the boiling point of water in normal atmospheric pressure. In this condition, the steam, released from the boiling water is of higher temperature. If any article placed in this system in such a condition, the high temperature destroys the microorganisms present in or on the article.

An autoclave is a metal vessel insulated by two metallic walls with a vacuum between the two walls. The upper end is open and can be closed by a lid to be tightened by some screws. At the bottom of the vessel, there is a heating coil, which is kept immersed in water. A metallic basket is fitted by 3–4 stands. The articles to be sterilized are placed in this basket so that water does not touch any article. At the top of the autoclave, attached to the lid, there are following devices:

i) A point regulated by a knob to release air or steam.
ii) A point for the adjustment of pressure.

iii) A meter showing the inside pressure.

2. Microscope:
3. Laminar flow cabinet

This is a chamber where microbial inoculation, isolation or any kind of transfer in microbiology or tissue culture is done. Laminar airflow transfer hoods are essential for any commercial operation involving such work.

They provide a sterile atmosphere to work with cultures. Air is forced through a HEPA (high efficiency particulate air) filter, located at the back of the hood that strains out particles as small as 0.3 micrometers. This airflow provides a sterile atmosphere in which the technician works. It is also provided with a UV (ultra-violet) lamp on the ceiling of the hood, which is put on for sometime (15 minutes) and then put off before the work is started.

4. pH meter:

A pH meter is an instrument that can indicate the pH i.e. acid / base status of a solution. There are several models of pH meter available in the market and each one comes with its own instructional manual.

pH measurement:

1. Connect the electrode to the pH meter and switch on the instrument.
2. Prepare 7.0 pH and 4.0 or 9.2 pH buffer solution using buffer tablets.
3. Wash the electrodes with distilled water and dry with a tissue paper.

4. Dip it in 7 pH buffer solution. Keep the temperature knob at 25°C. Set the function switch to pH position. The meter will give some reading near to 7.0 pH, say 6.8. Use STANDARDIZE control to make the reading exactly 7.00.

5. Remove the 7.0 pH buffer solution. Wash and dry the electrode as done before.

6. Dip the electrode in either 4.0 or 9.2 pH solution. If dipped in pH solution 4.0, it will give a reading say, 4.3. The error is 0.3 pH. Now use the SLOPE control (on back side of the instrument) to adjust the pH reading to 4.0. Now the pH meter is standardized and the controls are not to be disturbed.

7. Remove the solution, wash and dry the electrode.

8. Immerse the electrode in the sample solution and directly read its pH value panel.
Diagramatic view of the front page of a Digital pH Meter
5. **Conductivity meter:**

   Conductivity Meter is designed to measure the Electrical Conductivity of a solution. A conductivity meter has the following control panels:

   1. ON / OFF switch
   2. SET / CAL / READ button
   3. Digital panel meter (DPM)
   4. CELL CONSTANT
   5. RANGE

   ![Diagramatic view of the front of a Conductivity Meter](image)

**Operation procedure:**

1. Switch on the instrument
2. Select the SET / CAL / READ switch to "SET" and adjust the 'SET 100' control panel to read '100' on the digital panel meter.
3. Turn the range switch to extreme clockwise position.
4. Immerse the cell in the standard solution, the specific conductivity of which is accurately known, at the temperature of the solution.
5. Put the SET/CAL/ READ switch at 'CAL/READS' position and turn the RANGE switch to get the reading of the corresponding range. Now
adjust the CELL CONSTANT control so that the DPM display the right value of the specific conductivity of the standard solution.

6. Keep the RANGE switch to extreme position. Clean the cell by rinsing with distilled water, dip the cell in the solution, select the appropriate range and the DPM will display the specific conductivity of the solution.

Fermentation Glassware

The standard 250 ml Erlenmeyer flask is cheap and simple; most of the shaker tables designed to use these flasks although there are tables, which can be adapted to allow other shapes or bigger flasks.

Baffles have been used in shake flasks to assist in the OTR, as well as preventing vortex formation, but there are only really suitable for low-volume short-term fermentations because of splashing which leads to the cotton-wool plug becoming damp preventing free flow oxygen.

Different plugs can be made of cotton wool, glass wool, polyurethane foam, gauze or synthetic fibrous material. (An aluminium foil cup can sometimes be used in conjunction with these plugs). The plug has to be prevent airborne microorganisms from getting into the medium while at the same time allowing free flow of air into the flask, and for this reason it must not be allowed to become wet.

6.3.5 Stirred Fermenter
Basically, the stirred fermenter consists of a cylindrical tube a top-driven or bottom-driven agitator. The stirred fermenter with a top-drive assembly is the most commonly used fermenter because of its ease of operation, neat design, reliability, and robustness.

For smaller laboratory fermenters (bench-top), borosilicate glass is used as the cylindrical tank and a top plate of stainless steel clamped on. A motor is fixed above the top plate and is attached to the shaft. The motor can be uncoupled. The vessel, medium and probes are usually sterilized together in an autoclave, and minimizing the number of aseptic operations required (Fig. 6-2).

These glass vessels can vary in size from one liter to 20-liter capacity. The vessel itself will have a specific impeller design, baffles, an air sparger, and sample port.

The special "Rolls Royce" laboratory fermenter is constructed like hollow steel cylinder with either top or bottom drive and can be cleaned and sterilized in situ. These stirred fermenters can vary in volume from one liter to 100 litres capacity. Obviously they are more expensive than the glass vessels but they are more robust, reliable, and designed to a lifetime.
Often large samples or regular samples have to be taken for analysis during the fermentation. These volumes must be take into consideration when choosing a fermenter.

**Automation and Performance**

Bench-top fermenters are usually cheaper to purchase than the trolley-mounted or skid mounted fermenters. This is partly due to the fact that their instrumentation is often not as sophisticated as a laboratory or research fermenters. This latter have a sophisticated instrument control package for pH, temperature, and agitation, and this obviously costs more.

**Agitation and Aeration**

Most stainless steel fermenters are designed with bottom drive unit which is belt driven. This has several advantages:

It allows easy access to the top of the vessel and all the moving parts can be isolated and encased underneath the vessel, thus making it a safer piece of apparatus;

With the motor belt driving the agitator any spillage which occur will not fall onto the motor because it is not sited directly below the agitator shaft.

The agitation shaft should have a double mechanical seal which ensures that the medium does not leak out at the shaft housing. The agitation shaft will normally have two or thee impellers, each with four or six blades depending upon mixing requirements.

**The air supply is filtered by one of these filter types:**

Membrane type filters, containing a cellulose acetate, or nitrate membrane of known and consistent pore size, which therefore retains all particles larger than that pore size 0.2 mm or 0.45 æm pore size filters are suitable for most applications. These filters are relatively cheap, disposable, and usually readily inspected for blocking or fouling. They should be discarded after a fixed number of autoclave cycles. The manufacturer will normally indicate the number of cycles which can be withstood.
Packed-bed type filters, these filter have no uniform pore size and the mechanism of particle removal tends to be rather more complex. Typically, a filter housing is packed with glass wool or non-absorbent cotton wool. Such filters are vulnerable to compaction and to wetting which may allow channeling to occur. Sudden fluctuations in the pressure drop across the filter can cause release of particles or packing material under some circumstances. Such filters are readily constructed in-house, but their sole advantage is cheapness.

Cartridge filters are composed of a stainless steel or polycarbonate filter housing containing a removable filter element. The filter element is often composed of a hydrophobic material (e.g. PTFE) bonded in polypropylene. These filters can be steam sterilized in situ or autoclaved. They are initially more expensive than other types, but their reliability and durability make up for this.

The sterile air is then fed into the bottom of the fermenter dispersed by a sparger and thoroughly mixed into the medium by the agitation system.

Vent gases can be filtered by the same means, but one has to be aware of the risks of blockage due to carry-over of medium or foam-out. Such risks can be minimized by the use of a foam control system; either a mechanical system (offered as an option by some fermenter manufactures) or, more routinely, a system involving addition of some foam-suppressing chemicals (e.g. a silicone-based compound or polypropylene glycol). An efficient condenser fitted to the gas outlet will also reduce the likelihood of exit filter blockage.

Services

A number of essential services are required to run an efficient fermentation laboratory, namely air, steam, water, electricity, and if it is possible the gas. The level of each service required depends on the size of laboratory and the demands on that service. All services should be available 24 hours a day, seven days a week, for a dedicated fermentation laboratory; smaller laboratories housing only a small number of simple fermenters such as polyethylene cushions fermenter do not require this level of service.

Air
Air is required for many purposes in a fermentation laboratory, e.g. aeration of fermenters, operation of hydraulic autoclave doors, and calibration of gas analysis equipment.

Laboratory fermenters are often supplied with integral air pumps. Individual air pumps can be purchased to supply air vessels which do not have an integral air supply and which are situated in laboratories without service air. The ideal pump is the diaphragm pump. These pumps vary greatly in their capacity and should be purchased according to the demand of the vessel. It is essential that the pumps be oil free and be suitable for prolonged continuous usage. Diaphragm pumps are relatively cheap and are useful if only one or two vessels require an air supply.

For larger laboratories, a more sophisticated air supply is required and a compressor should be purchased. The type and size of compressor selected depends on the function it is to have in laboratory. The compressor should be able to meet the current demands of the laboratory with additional capacity to allow subsequent growth.

Fermentation processes require particle-free clean air that meets food grade standards, so the compressors utilized must be oil-free units. Beware of compressors which are claimed to produce oil-free air; these are not oil-free compressors and should be avoided since even when functioning well they can allow oil to escape into the system, leading to fouling of lines and contamination of the fermentation. If such a system is already installed, the air lines must have seizable protective filters downstream of the compressor, which are regularly cleaned and serviced.

To ensure a constant supply of air at all times it is advisable to operate two compressors so that if one fails for any reason, the other unit will cut in and maintain the supply going. It is necessary to ensure that each compressor is used on alternate days so that the demand on each machine is equal. Incorporating an air receiver in the supply line decreases the demand on the compressor and also allows condensate to be removed from the supply.

If the compressor is to be situated in the laboratory, acoustic hoods should be fitted to reduce the noise level. Ideally the compressor(s) will be housed in a room situated adjacent to the fermentation laboratory.
The air supply is normally taken from the compressor to the fermenter via network of pipes. The pipework should be made of a non-corrosive material with a smooth interior to prevent build-up of dirt. Half-inch steel piping is excellent for this purpose and is also relatively cheap. Pressure-reducing valves must, therefore, be installed downstream of the compressor along with oil and water traps (as compressed air expands in the lines a small amount of water is formed 0.025 g H₂O per liter air at 25°C). All reducers and traps should be regularly cleaned and serviced. All connections made in the lines must be able to withstand to pressure at which the air is to be delivered (frequently 1-2 bar g, but this depends on the fermenter(s) used). It is, therefore, necessary to have the systems of pipework installed by qualified plumbers.

**Steam**

Steam is required in a fermentation laboratory for sterilizing fermenters, controlling temperature in large vessels, autoclaving, and supplying steamers. Each fermenter should have its own steam supply which can be isolated from the vessel by means of a gate valve. The steam supply should be as dry as possible, and all lines in the laboratory should be well lagged to help prevent the formation of condensate. Lagging is also important for the protection of laboratory personnel. The lagging should be covered with an aluminium casing to give a finish that looks more presentable, is easily cleaned and prevents the lagging material getting damp.

As with the air supply it may be necessary to incorporate pressure reducers in the lines. These should be carried out by qualified personnel.

**Water**

A fermentation laboratory requires a constant supply of water to service fermenters, downstream processing equipment, and autoclaves, as well as for analytical purposes.

Mains water is normally connected to the service inlet on the fermenter chassis. Manufacturers will indicate the maximum inlet pressure a particular model of fermenter can cope with. This is often very different for different vessels, e.g. a small lab fermenter is generally serviced by water supplied at 1 bar g whereas a small-scale vessel may require up to 3 bar g. The mains water inlet pressure must be sufficient to meet the demands of the laboratory; if necessary, pressure
reduction valves can be fitted on line to each vessel to allow a range of a different demands to be serviced.

The laboratory must also be supplied with good-quality water, for medium preparation and in areas where the local supply water is hard it may be necessary to install a deionizing system.

Water is also required for the basic laboratory chores of washing glassware, etc., and hand washing.

**Electricity**

Electricity is required for lighting and for supplying power to complex array of machinery and instrumentation found in a laboratory.

Many items of equipment in a fermentation laboratory draw amounts of power, e.g. small-scale vessels, autoclaves, and downstream processing equipment. It is, therefore, necessary in dedicated fermentation laboratories to have a supply of three phase electricity. Installation of three phase supplies can be expensive; it is advisable, therefore, to have a supply in the laboratory and to take off lines as and when required.

Every fermenter in the laboratory will require a number of electrical sockets supplying power to fermenter, pump and additional equipment, such as portable pH and O₂ meters. A bank of six sockets per fermenter is ideal for this purpose. For larger fermenters requiring a three-phase supply it is still very useful to have a bank of single phase sockets which should be installed alongside the three-phase supply.

**Gas**

Natural gas should be supplied to the general laboratory area usage. Speciality gases, e.g. carbon dioxide, oxygen, and calibration gases, are usually provided in the form of compressed gas cylinders which should be secured with safety straps.

**Fermenters**
A well-equipped fermentation laboratory requires substantial capital investment for the purchase of fermenters and a wide range of ancillary equipment (see ANNEX 2.).

6.5 Ancillary Equipment

**Autoclaves**

The size and type of autoclave purchased will depend on the number of fermenters being serviced. A standard size autoclave, e.g. 450 x 800 x 1200 mm chamber dimension, will be sufficient to deal with ancillary purposes, e.g. sterilizing glassware, or preparation of small quantities of media.

**Bench-top or portable autoclaves**

Bench-top or portable autoclaves are useful for sterilizing small items, e.g. bottles, a small number of 500 ml conical flasks. Pressure cookers can be used for this purpose, but the life span of the portable autoclave is generally longer, because it is more robust in construction.

**Standard Autoclaves**

Standard Autoclaves small volumes from 1 to 10 litres

**Incubators, Shakers**

Incubators are required for the cultivation of stock cultures and production of inocula.

**Ovens**

Two types of oven are useful in fermentation

(i) **Hot air ovens**: Can be used for drying glassware or dry weights and sterilizing.

(ii) **Microwave Ovens**: Are used for dry weights, drying glassware, melting agar etc.

**Pumps**
At laboratory scale, liquid pump is achieved almost exclusively by means of peristaltic pumps. The choice of pumps will depend on the application.

**Minipumps**

These fixed-speed pumps are normally used for addition of acid/alkali, antifoam linked to a pH/antifoam controller.

**Larger Pumps**

Lager pumps, used for example for nutrient addition in continuous or fed-batch culture, generally have clamps to hold tubing on the pump heads. Such pumps, which take tubing of up to 8 mm bore, can be either fixed speed or variable speed, manual or auto control.

If is often necessary to sterilize larger volumes of medium separately from the fermentation vessel, then pump the sterile medium into the pre-sterilized vessel. For such purposes, a high-speed pump, capable of delivering at least 3-5 liter min⁻¹ using standard 5-6 mm bore tubing, is very useful.

When buying a pump, it is important to think carefully about the intended flow rate range. Due to nature of electric motors, pumps are most accurate at high speeds and least accurate at very low settings. On the other hand, tube life is much reduced at high speeds.

**Medium Reservoirs**

Particularly in fed-batch and continuous culture it is essential to have a number of medium supply vessels. Such vessels must be able to withstand repeated autoclaving. The volume of vessels required (and the number) will depend on the experimental programme, e.g. a 10 litre fermenter operated at a dilution rate of 0.1 h⁻¹ will use 24 litres per day, so 5 litre reservoirs would be inappropriate. In practice, glass (heat resistant) and polycarbonate vessels are superior to glass because they are lighter, not so dangerous if dropped, and to show distinct signs of wear before they fall, whereas an undetected fault in a glass vessels can cause a sudden failure. After autoclaving, allow vessel and contents to cool before removing them. Medium components likely to be affected by heat should be filter sterilized and added aseptically later.
**Cooling System**

The medium after sterilization in the pilot plant fermenters must be cooling before inoculation during two or four hours.

**"O" - Rings**

O-rings are in constant use in a fermentation laboratory. They are used as the compressible material when a seal is made between glass and metal or between metal and metal. O-Rings are usually composed of nitrile or butyl rubber, sometimes of silicone. Remember that items such O-rings have a finite life span dependent upon how often they are autoclaved, and whether or not they are deformed by over-compression; they will thus have to be carried out as part of the regular maintenance programme.
Project details:

Installed Capacity
The project suggested is with an installed capacity of production of biofertilizers (N, P and K biofertilizer) is 50000kg powder based and 50000ltr liquid formulation, Biopesticides- 100000 kg Wettable Power and 50,000 litters liquid annually considering 300 working days. The suggested income contribution are as follows: Biofertilizer (40%), WP formulation of bio pesticides (40%) and biopesticide liquid formulation (20%).

Land & Building
The requisite of any biopesticide and biofertilizer production unit comprise appropriate location, land, building space and design, equipments, machinery, other laboratory material, working capital and personals. Facilities for the propaganda are also part of the unit.

1. Location: It should represent agroclimatic zone and located at the center of each such zone for supply of biopesticide and biofertilizer to farming community. Buffer zone should be there to reduce chances of i). Contaminantion during the production and quality control process ii). Test insects/pathogen moving from laboratory to field crop iii). Ill effects drift of field used chemical/contaminants on mother cultures.

2. Land: It should have adequate irrigation facilities and should have some properly leveled cultivable land for host plants and carrying out pilot experiment around the laboratory.

3. Building: It is the most important infrastructure as compared with other long-term assets for years. Planning for space required for different biopesticide/biofertilizer units and sub units is a crucial and feasible consideration as per target for production of biopesticides.

4. Space required: Considering production of 10,0000 litter biofertilizer, 100000 kg WP formulation of biopesticides and 50000 litres fungal biopesticides per annum, subsequent requirements have been given below:

<table>
<thead>
<tr>
<th>#.</th>
<th>Particulars</th>
<th>Construction type</th>
<th>Number</th>
<th>Total space (sq. ft.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unit office room</td>
<td>RCC</td>
<td>1</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>Culture preparation/dark room</td>
<td>RCC</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Media preparation and</td>
<td>RCC</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>No.</td>
<td>Room Description</td>
<td>Type of Construction</td>
<td>No.</td>
<td>Area (sq ft)</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------------------------------------</td>
<td>----------------------</td>
<td>-----</td>
<td>--------------</td>
</tr>
<tr>
<td>4</td>
<td>Autoclave room</td>
<td>RCC</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>Fermentation room</td>
<td>RCC</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>6</td>
<td>Formulation room/Mixer room</td>
<td>Close shed</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>8</td>
<td>Quality control lab.</td>
<td>RCC</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>12</td>
<td>Store rooms</td>
<td>Close shed (raw material)</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>13</td>
<td>Store rooms</td>
<td>RCC (finished goods material)</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>13</td>
<td>Toilet (Ladies and Gents)/cleaning &amp; washing area</td>
<td>Close shed</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>16</td>
<td>Water storage tank</td>
<td>RCC (15000 Itr. capacity)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Open land</td>
<td>--</td>
<td>--</td>
<td>950</td>
</tr>
</tbody>
</table>

**Total area needed:** 4330 sq feet

**RCC construction area needed:**

**Closed shade area needed:**

**Total area needed:** 4330 sq ft

**Super quality RCC construction cost:** @ Rs.------- x 2930 sq. ft. = Rs.--------

**Closed shed construction cost:** @ Rs.-------- sq.ft. x 1400 sq.ft. = Rs.-------

**Open land cost:** @ ------Rs. per sq.ft. x 950 sq.ft. = Rs.------------

**Total cost of building = Rs.**  ---------------------

**5. Design:** It should be close type with impressive entrance at middle of length of “H” or suitable type outer design with open space for pot culture at the center of the building. It should facilitate sufficient natural light ventilation, climate control provisions devices like air, conditioners, 5 and 15 ampere plugs in each room and halls, safety system to avoid damages by short circuit, adequate hot and cold water supply with working generator a alternative to power cut problem, leak proof ceiling and damp proof floors and walls, glazed tiles or polyvinyl mat floors, glass windows and wide (3’) doors with auto – shutters, three phase power supply for heavy machinery, well drainage system, 75 cu.m. water...
storage tank(s) and ceiling and exhaust fans fitting facilities. Office should be nearer to entrance to reduce chances of contaminants. Golden duranta border fencing around the building, lawn, and ornamentals in front of the building adds in built up the building. Non-deciduous shrubs and free plantation help in climate control, if planted around the building.

**Infrastructure:**
The required machinery & equipments are depicted in table-2.

<table>
<thead>
<tr>
<th>Sr</th>
<th>Machinery/instrument</th>
<th>No</th>
<th>Capacity</th>
<th>Prices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Autoclave</td>
<td>1</td>
<td>500kg Horizontal</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Autoclave</td>
<td>1</td>
<td>100kg Verticle</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Autoclave</td>
<td>1</td>
<td>50 kg Verticle</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Fermenter</td>
<td>1</td>
<td>2000 ltr Fabricated</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Fermenter</td>
<td>1</td>
<td>500 ltr Fabricated</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Laminar air flow</td>
<td>2</td>
<td>size 4'x2'x2'</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pan Balance</td>
<td>1</td>
<td>5000 gms, accuracy-0.0001 gm (0.1mg)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pan Balance</td>
<td>1</td>
<td>120 Kg, accuracy-0.0001 gm (0.1mg)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PH Meter</td>
<td>1</td>
<td>electrode, range 0 to 14 pH.</td>
<td>Accuracy – 0.01</td>
</tr>
<tr>
<td>10</td>
<td>Water Distillation Unit</td>
<td>1</td>
<td>20 L/hour</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Air conditioners</td>
<td>3</td>
<td>1.5 Ton</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Trinocular Microscope</td>
<td>1</td>
<td>4x,10x,40x,100x oil immersion Eyepiece WF 10x20mm field of view.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Air Curtain</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Lab. Hot Air Oven</td>
<td>1</td>
<td>Temp – 50 to 250 Deg. Cent</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Bacteriological Incubator</td>
<td>1</td>
<td>temp – ambient+5 to 60 deg.C</td>
<td>chamber size 60x60x60 cms.</td>
</tr>
<tr>
<td>16</td>
<td>Rotary Shaker</td>
<td>4</td>
<td>48x48' platform, to hold 81 flasks of 1 litre or 100 flasks 500 ml</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Heavy Duty powder Mixer/blender</td>
<td>1</td>
<td>1000 kg/hr</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Magnetic stirrer (MLH)</td>
<td>1</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>Cyclomixer</td>
<td>1</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
The total machinery, equipment and glassware cost about Rs. 38.17 lakhs.

**Raw Materials**
The required raw material is media, chemicals, carrier materials, rice for solid state fermentation as a carrier for fungal pesticide. The raw materials needed for production of 2.5 lakh ltr/kg will cost Rs.37.5 lakhs annually.

**Utilities**

**POWER:**

**POWER CONSUMPTION**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the Equipment</th>
<th>Capacity</th>
<th>Power In</th>
<th>Power Consumption Units Per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Autoclave Horizontal</td>
<td>chamber, size 600x600x1200 mm</td>
<td>18 kw</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>Autoclave Vertical (2)</td>
<td>inner size 55x75 cms (vol:178 lit)</td>
<td>10 kw</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Monopan Balance</td>
<td>(200 gms, accuracy-0.0001 gm(0.1mg)</td>
<td>0.00025kw</td>
<td>0.00075</td>
</tr>
<tr>
<td>6</td>
<td>Pan Balance</td>
<td>120 Kg, accuracy-0.0001</td>
<td>0.00025kw</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Specifications</td>
<td>Power</td>
<td>Capacity</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>7</td>
<td>PH Meter</td>
<td>electrode, range 0 to 14 pH, Accuracy – 0.01</td>
<td>0.00025k</td>
<td>0.001</td>
</tr>
<tr>
<td>8</td>
<td>Laminar Flow Apparatus</td>
<td>size 4'x2'x2'</td>
<td>1.5 kw</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>Water Distillation Unit</td>
<td>20 L/hour</td>
<td>8.0 Kw</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>Air conditioners</td>
<td>1TR</td>
<td>3 kw</td>
<td>24</td>
</tr>
<tr>
<td>11</td>
<td>Trinocular Microscope</td>
<td>4x,10x,40x,100x oil immersion, Eyepiece WF 10x20mm field of view.</td>
<td>0.00025k</td>
<td>0.001</td>
</tr>
<tr>
<td>13</td>
<td>Air Curtain</td>
<td></td>
<td>0.75 kw</td>
<td>1.5</td>
</tr>
<tr>
<td>14</td>
<td>Lab. Hot Air Oven</td>
<td>Temp – 50 to 250 Deg. Cent</td>
<td>2.5 kw</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>Bacteriological Incubator</td>
<td>temp – ambient+5’ to 60 deg.C, chamber size 60x60x60 cms.</td>
<td>0.75 kw</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>Rotary Shaker</td>
<td>48x48’ platform, to hold 81 flasks of 1 litre or 100 flasks 500 ml</td>
<td>1.5 kw</td>
<td>36</td>
</tr>
<tr>
<td>17</td>
<td>Heavy Duty Powder Mixer</td>
<td>100 kg/hr</td>
<td>2.25 kw</td>
<td>13.5</td>
</tr>
<tr>
<td>18</td>
<td>Fermentor</td>
<td>2500 Ltr.</td>
<td>3.75 kw</td>
<td>9</td>
</tr>
<tr>
<td>19</td>
<td>Fermentor</td>
<td>500 Ltr.</td>
<td>2.75 kw</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>Magnetic stirrer (MLH)</td>
<td></td>
<td>0.3 kw</td>
<td>0.6</td>
</tr>
<tr>
<td>21</td>
<td>Cyclomixer</td>
<td></td>
<td>0.00025k</td>
<td>0.005</td>
</tr>
<tr>
<td>22</td>
<td>Boiler and Softner</td>
<td></td>
<td>3.75 kw</td>
<td>2.25</td>
</tr>
<tr>
<td>23</td>
<td>Deep freezer and water cooler</td>
<td></td>
<td>4.5 Kw</td>
<td>108</td>
</tr>
</tbody>
</table>
25 Tube lights & Fans

10 tubes
6 Fans

0.64 kw 0.16

The plant will require power of 148 KW per day and the total power cost will be around Rs.4.5 lakhs per year.

**Water:**
The total water requirement of the unit would be 2000 lits per day, and the total cost will be 1 lakhs per year.
The total costs towards utilities will be about Rs.5.5 lakhs per year.

**Manpower Requirement**
The project provides employment as follows.

<table>
<thead>
<tr>
<th>#</th>
<th>Particulars</th>
<th>No.</th>
<th>Salary per annum (Rs. In Lakhs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scientist</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Technical assistant</td>
<td>2</td>
<td>1.44</td>
</tr>
<tr>
<td>3</td>
<td>Semiskilled workers</td>
<td>5</td>
<td>1.80</td>
</tr>
<tr>
<td>4</td>
<td>Daily wages workers (300 working days@250/day)</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Total 11.84

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fringe benefit @ 20% + 5% annual rise</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Total 10.60

The daily wages workers for filing packing and other work will be procured as needed.

**Working Capital Requirement**
The project requires two months stock of packing material, one month’s utilities, one month’s salaries, two months for work in process, one month's finished stock and one month's bills receivable. The working capital requirement works out to Rs.11.40 lakhs.
**Preliminary & Pre-operative Expenses**
The total preliminary & pre-operative expenses amount around Rs.13 lakhs and these are the costs towards technology, project report preparation, traveling, salaries, interest, trial production, deposits & contingencies.

**Project Cost and Means of Finance:**

<table>
<thead>
<tr>
<th>#</th>
<th>Particulars</th>
<th>Amount (Rs. In Lakhs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Project cost</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Land and building</td>
<td>RENTAL</td>
</tr>
<tr>
<td>B</td>
<td>Plant and machinery</td>
<td>33.43</td>
</tr>
<tr>
<td>C</td>
<td>Miscellaneous fixed assets</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Preliminary &amp; preoperative expenses including deposits total fixed capital</td>
<td>13.00</td>
</tr>
<tr>
<td>E</td>
<td>Working capital margin</td>
<td>11.4</td>
</tr>
</tbody>
</table>

**Total**

<table>
<thead>
<tr>
<th>#</th>
<th>Means of Finance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Promoters contribution</td>
<td>14.5</td>
</tr>
<tr>
<td>B</td>
<td>Term loan</td>
<td>29.0</td>
</tr>
<tr>
<td>C</td>
<td>Subsidy</td>
<td>14.5</td>
</tr>
</tbody>
</table>

**Annual Operating Results:**

<table>
<thead>
<tr>
<th>#</th>
<th>Particulars</th>
<th>Amount required (Rs. In Lakhs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cost of Production</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Raw material</td>
<td>25.00</td>
</tr>
<tr>
<td>b.</td>
<td>Utilities</td>
<td>5.50</td>
</tr>
<tr>
<td>c.</td>
<td>Salaries</td>
<td>10.60</td>
</tr>
<tr>
<td>d.</td>
<td>Packaging material</td>
<td>24.5</td>
</tr>
<tr>
<td>e.</td>
<td>Repairs, maintenance, insurance</td>
<td>1.0</td>
</tr>
<tr>
<td>f.</td>
<td>Depreciation</td>
<td>0.7</td>
</tr>
<tr>
<td>g.</td>
<td>Telephone &amp; post</td>
<td>0.2</td>
</tr>
<tr>
<td>h.</td>
<td>Selling expenses</td>
<td>10.0</td>
</tr>
<tr>
<td>i.</td>
<td>Preliminary &amp; preoperative expenses</td>
<td>13.00</td>
</tr>
<tr>
<td>j.</td>
<td>Interest</td>
<td></td>
</tr>
</tbody>
</table>

**Total**

| B  | Net sales (1st year)                                                       | 120.00                       |
| C  | Profit before tax                                                          | 6.00                          |
SWOT Analysis:
1. Strength:
The cropping pattern in India favors multiplication and carry over of the pests. Hence, pesticide consumption is also high. As such, there is wide scope for promoting the use of biopesticides.

2. Weakness:
The shelf life of bioagents is very small, as such its availability in market to the producers should be in the vicinity. As the concept being quite new, it is necessary to support the manufacturing units by providing production subsidy in the beginning.

3. Opportunities:
With open economy, there is vast scope for export of agro-products, which needs to be residue free. This opens a door for large-scale use of biopesticides. Due to increasing cropping intensity, the overlapping generations of pest occurs. Use of bioagents thus has wide scope.

4. Threats:
Competition from chemical pesticides manufactured by multinationals, which are financially very strong.
CONTACT DETAILS

BIOMATE INDIA (AN ISO 9001 CERTIFIED COMPANY)

Registered Office: A-5/45, Sector-15 Rohini New Delhi - 110089, India
Factory Address: 436, Shahabad, Bawana Rd, Near Delhi Technological University (DCE), Rohini, Delhi - 110042, IN

Internet details: Web site: www.biomateindia.com, E-Mail: salesbiomate@gmail.com
Cell nos Tele fax: +91 9818648066, 9312130119, 11 27872365.